



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> :  C12N 15/80, 1/15 // (C12N 1/15, C12R 1:665, 1:685, 1:77, 1:885)		A1	(11) International Publication Number: <b>WO 98/45455</b>  (43) International Publication Date: 15 October 1998 (15.10.98)
(21) International Application Number: PCT/EP98/01914			naarseweg 64, NL-2333 AL Leiden (NL). GOUKA, Rober-
(22) International Filing Date: 24 March 1998 (24.03.98)			tus, Johannes [NL/NL]; Unilever Research Vlaardingen, Olivier van Noortlaan 120, NL-3133 AT Vlaardingen (NL).
(30) Priority Data: 97201022.7 7 April 1997 (07.04.97) EP (34) Countries for which the regional or international application was filed: NL et al.			DE GROOT, Marcellus, Johannes, A. [NL/NL]; Unilever Research Vlaardingen, Olivier van Noortlaan 120, NL-3133 AT Vlaardingen (NL). HOOYKAAS, Paul, Jan, J. [NL/NL]; Rijks Universiteit Leiden, Instituut Moleculaire Plantkunde, Clusius Laboratorium, Waskenaarseweg 64, NL-2333 AL Leiden (NL).
97204062.0 22 December 1997 (22.12.97) EP (34) Countries for which the regional or international application was filed: NL et al.			(74) Common Representative: UNILEVER N.V.; Patent Division, P.O. Box 137, NL-3130 AC Vlaardingen (NL).
(71) Applicant (for all designated States except AU BB CA GB IE KE LK LS MN MW NZ SD SG SZ TT UG US): UNILEVER N.V. [NL/NL]; Weena 455, NL-3013 AL Rotterdam (NL).			(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).
(71) Applicant (for AU BB CA GB IE KE LK LS MN MW NZ SD SG SZ TT UG only): UNILEVER PLC [GB/GB]; Unilever House, Blackfriars, London EC4P 4BQ (GB).			
(72) Inventors; and (75) Inventors/Applicants (for US only): BEIJERSBERGEN, Alida, Godelieve, M. [NL/NL]; Unilever Research Vlaardingen, Olivier van Noortlaan 120, NL-3133 AT Vlaardingen (NL). BUNDOCK, Paul [NL/NL]; Rijks Universiteit Leiden, Instituut Moleculaire Plantkunde, Clusius Laboratorium, Wasse-			Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>

(54) Title: AGROBACTERIUM MEDIATED TRANSFORMATION OF MOULDS, IN PARTICULAR THOSE BELONGING TO THE GENUS ASPERGILLUS

(57) Abstract

The invention relates to *Agrobacterium* mediated transformation of moulds comprising species of the fungal sub-divisions *Ascomycotina*, *Basidiomycotina*, *Deuteromycotina*, *Mastigomycotina*, and *Zygomycotina*. Examples demonstrate the transformation of *Aspergillus awamori* (both protoplasts and conidia), *Aspergillus nidulans*, *Aspergillus niger*, *Colletotrichum gloeosporioides*, *Fusarium solani pisi*, *Neurospora Crassa*, *Trichoderma reesei*, *Pleurotus ostreatus* and *Agaricus bisporus* (all conidia), and *Fusarium graminearum* (both conidia and rehydrated freeze dried ATCC material). Especially for *Aspergillus awamori* the transformation frequency is much higher than with conventional mould transformation techniques. It has further been found that not only one expressable gene can be introduced into these moulds, but even multiple copies of such gene, which, moreover, can be targeted e.g. in the chromosomal *pyrG* locus, as exemplified for *A. awamori*. These multiple copies can be of a gene encoding a desired, homologous or heterologous, protein.